Constructing and comparing networks of water stress response across Durum wheat cultivars

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Wheat is an important crop and Durum wheat is widely grown in Mediterranean climates for the production of pasta ,bread and other products. Drought is the major abiotic limitation to crop yield with significant agricultural, economic, political and social impact, especially since durum wheat is mainly farmed under rain-fed environments. In this work we propose to identify and compare the networks of gene expression responses to water stress between three cultivars and breeding lines of Durum wheat known to respond differently to drought in multiple field trials. The objective is to identify key genes, networks and metabolic pathways differentially affected by the stress response.

A progressive drought stress experiment
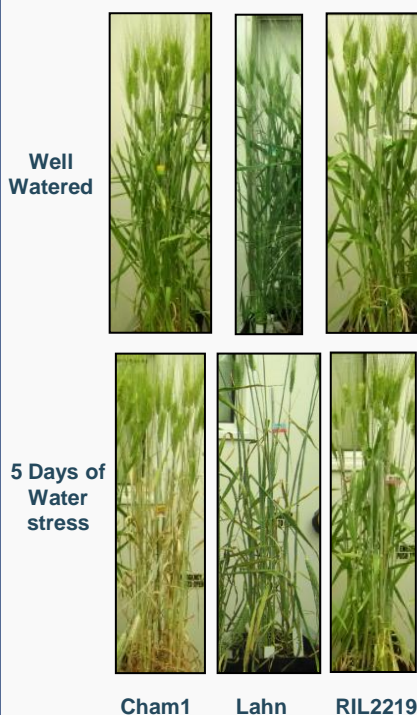
Recently, a controlled environment experiment on the response to water stress was conducted at Rothamsted Research (UK). The effects of 5 days of progressive water stress on flag leaf gene expression was studied at a global genome scale using Affymetrix micro-arrays, in three cultivars of durum wheat, namely Cham1, Lahn and RIL2219.

Cham1 is a drought resistant variety widely grown in the Mediterranean basin and Lahn is a high yielding variety from Syria, which performs well under well-watered conditions but is susceptible to changes in temperature and water availability. RIL2219 is one of the 114 Recombinant Inbred Lines (RIL) originating from the cross between Cham1 and Lahn.

Well Watered

5 Days of Water stress

Cham1   Lahn   RIL2219

At the phenotypical level, the difference between the three cultivars after 5 days of water stress is obvious and can be accurately measured when looking at several physiological variables such as the Relative Water Content , the concentration of ABA or the stomatal conductance. Lahn is thus the most susceptible parent to water stress, followed by the second parent Cham1 and finally RIL2219 being the most resistant to the stress.
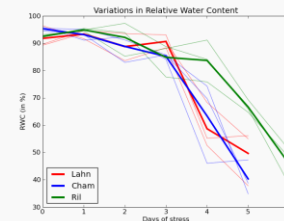
After standard normalisation and ANOVA, 18 642 probe sets are selected to be significantly affected by the stress when compared to well watered controls. The transcriptome of the three cultivars is highly correlated; the correlation of the mRNA concentrations between cultivars is above 94% even after 5 days of water stress.

Pure statistical analysis techniques were not sufficient to dissect the complex relationships between the genes and more information is needed to understand the process of regulation.
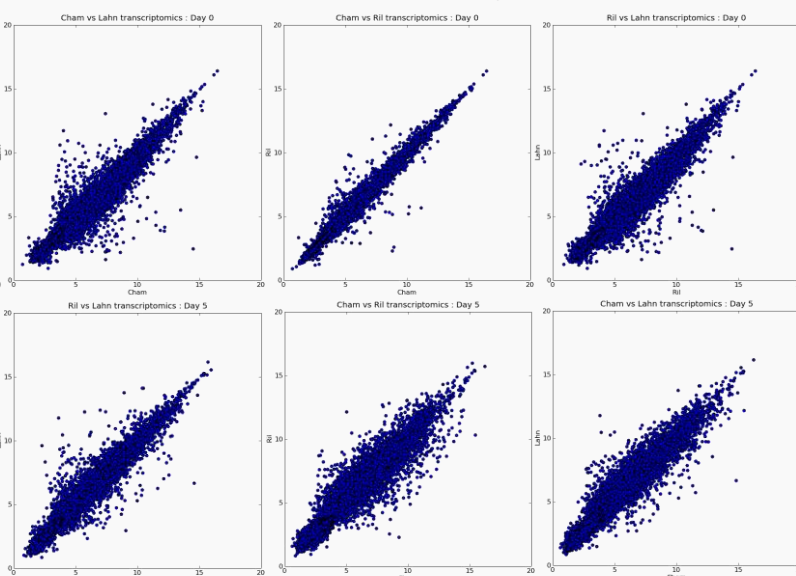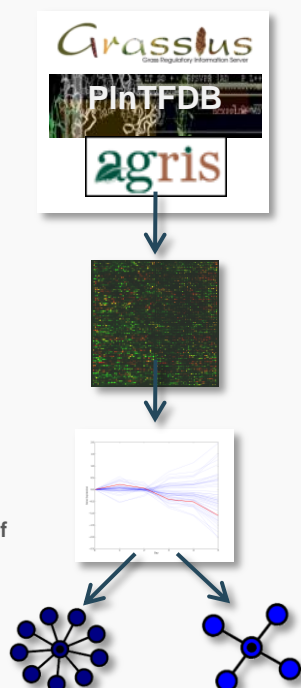
Networks of stress response

We have first identified the probes on the wheat Affymetrix chip that correspond to potential Transcription Factors (TFs). We used BLAST to compare the ESTs sequences to three plant TF databases (Grassius, PlntDB, AGRIS).

Grassius
PlnTFDB
agris

In a second step, the micro-array data have been analysed using ANOVA and TFs whose expression profile differ significantly from the well watered condition were selected.

Then we have been searching for possible regulated genes (blue lines) for each TF (red line) by looking at the level of co-expression. A regulation is assumed for unsigned Pearson's correlation coefficient above 0.9. Resulting co-expression networks correspond to units of regulation, also known as regulons.

Regulons are merged when they have genes in common to form more complex networks of stress response which ones can then be compared among cultivars.

Example of early stress response

Networks of early stress response have been constructed in the three cultivars. They contain TFs whose activity differ significantly from the well watered condition after one day of drought.

Cham1   Lahn   RIL2219
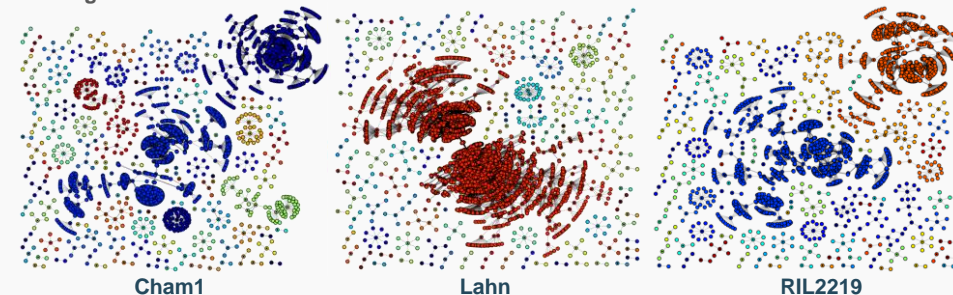
The most noticeable difference between the three networks is that for Lahn a much higher number of TFs and regulated genes has been found. However, in terms of topological properties, the networks appeared to be very similar. The main component of the Lahn network is really massive compare to the other cultivars since it contains more than five times more TFs and regulated genes. Network analysis have also shown the significantly higher cohesiveness and hub likeness of this component.

Network	Cham1	Lahn	RIL2219
# TF	211	358	201
# orphan TF	26	29	25
# Regulated Genes	3287	6766	2281
# Components	121	124	111

| Main component	Cham1	Lahn	RIL2219
# TF	31	218	39
# Regulated Genes	1292	6437	1068
Average Degree	3.36	6.19	2.72
Clustering Coef	0.26	0.37	0.14
Diameter	13	27	18

Conclusions and future work

In this study, we have identified a massive regulon in Lahn, revealing an early but co-ordinate response to stress in this cultivar. Conversely, we have seen that both drought resistant parent Cham1 and RIL2219 show conservative responses compared to the drought susceptible parent. In the future, we intend to study in more details the main units of regulations, for example by clustering the networks and computing their functional enrichment.

Reference

Habash et al. 2009 Genomic approaches for designing durum wheat ready for climate change. J. Exp Bot. 60,2805-2815